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HIGH AND LOW DENSITY LIPOPROTEIN CHOLESTEROL IN RELATION TO WHOLE BLOOD VISCOSITY

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We measured whole blood viscosity in a normal population and analyzed results in relation to packed cell volume (hematocrit, PCV), fibrinogen, white blood cell count (WBC), platelet count and plasma lipids including total cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc). A strong overall correlation was seen between WBV and PCV ($r = 0.78$, $p < 0.001$). Significantly positive

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correlations were found between WBV and cholesterol ($r = 0.22$, $p < 0.001$), triglycerides ($r = 0.14$, $p < 0.001$) and LDLc ($r = 0.21$, $p < 0.001$). A significant negative correlation was found between HDLc and WBV ($r = -0.20$, $p < 0.001$). However, analysis by sex showed only the correlation of LDLc was significant for both men and women. These data confirm a correlation between WBV and LDLc. WBV deserves further study as a potentially reducible risk factor in groups known to be at risk for heart disease because of abnormally high levels of LDLc.

ABSTRACT

PURPOSE: To measure whole blood viscosity in a normal population and analyze results in relation to packed cell volume, (hematocrit, PCV), fibrinogen, white blood cell count (WBC), platelet count and plasma lipids including total cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc).

METHODS: Conventional assays for all blood measurements. WBV measured under disaggregating conditions with a disposable, porous bed viscometer.

RESULTS: A strong overall correlation was seen between WBV and PCV ($r= 0.78$, $p<0.001$). Significantly positive correlations were found between WBV and cholesterol ($r=0.22$, $p<0.001$), triglycerides ($r=0.14$, $p<0.001$) and LDLc ($r=0.21$, $p<0.001$). A significant negative correlation was found between HDLc and WBV ($r= -0.20$, $p<0.001$). However, analysis by sex showed only the correlation of LDLc was significant for both men and women.

CONCLUSION: These data confirm a correlation between WBV and LDLc. WBV deserves further study as a potentially reducible risk factor in groups known to be at risk for heart disease because of abnormally high levels of LDLc.

KEY WORDS: Blood viscosity, lipoproteins, arteriosclerosis, hematocrit, gender, cholesterol, heart disease.

INTRODUCTION

The role of blood viscosity in the pathogenesis of cardiovascular disease has been the study of several recent epidemiological studies (1-8). In these studies abnormal hemorheologic parameters have been shown to be associated with hypercholesterolemia. Moreover, a recent report on the blood rheology after LDL apheresis using dextran sulfate cellulose absorption (9) demonstrated that selective extracorporeal LDL cholesterol elimination resulted in a 32% fall in whole blood viscosity (WBV), standardized to a hematocrit of 45% suggesting that LDLc reduction may influence WBV.

Increases in serum cholesterol and triglycerides that correlate with increased plasma viscosity may be due to the direct effect of lipoproteins on plasma viscosity (3). Plasma with chylomicronemia similarly may elevate plasma viscosity. A multivariate analysis of three major cardiovascular risk markers, cholesterol, blood pressure, and smoking showed that these risk factors had both independent as well as additive effects on blood viscosity (4). In the present study we have investigated the relation between whole blood viscosity and plasma lipoproteins. In addition to measurements of total cholesterol and trigylcerides, we performed measurement of high density lipoprotein cholesterol (HDLc) and calculation of low density lipoprotein cholesterol level (LDLc).

The results to be presented confirm that there is a statistically significant correlation between cholesterol and triglycerides with whole blood viscosity. The results further show that this relation is mainly due to the influence of LDLc cholesterol.

METHODS

I. SUBJECTS

The specifics of the selection and interviewing of subjects has been described previously (10,11). Briefly, the evaluation effort of the Pawtucket Heart Health Program includes biennial risk factor surveys. Households in Pawtucket, RI and in a comparison city in southeastern Massachusetts are randomly selected and visited by a trained surveyor. One resident aged 18-64 is then randomly selected from each household for interview concerning cardiovascular disease risk factors, blood pressure measurement and phlebotomy. We studied 982 individuals. The average age was 41.3 ± 14.5 (range 18-73). There were 43.5% males and 56.7% females. This population was 89.5% Caucasian and consisted of 91.3% non-smokers.

II. LABORATORY MEASUREMENTS

Total cholesterol (12), triglyceride (13) and high density lipoprotein cholesterol (14) measurements were performed on serum. Low density lipoprotein cholesterol (LDLc) was calculated using the following formula:

$$LDLc = \text{total cholesterol} - \frac{\text{triglyceride}}{5} - HDLc$$

Blood cell parameters and whole blood viscosity (WBV) were measured on whole blood drawn into a test tube containing K_3EDTA. White blood cell count, red blood cell count, hemoglobin, hematocrit, and platelet count were measured on a Coulter T540 (Coulter Electronics, Hialeah, FL). RBC indices were calculated using standard formulae. Fibrinogen levels were measured on an automated Coag-a-mate x 2

(General Diagnostics) using a thrombin-time method. Whole blood viscosity was measured using a porous bed viscometer (PBV)(15) after warming the blood and the viscometers for 30 minutes at 37°C. Using the PBV, the time in seconds required for 0.1 ml of blood to pass through the porous bed is measured. Using identical devices the flow time for 0.1 ml of 10.0 centipoise calibration liquid at 37°C to pass through the porous bed is 27.5 ± 0.5 seconds. Therefore, each second of measured flow time is equivalent to 0.364 ± 0.06 centipoise (15). Since one centipoise is essentially equivalent to one milliPascal·second (mPa·sec), we have converted our measurements as follows:

Flow time in seconds x 0.364 = whole blood viscosity in mPa·sec.

Blood collected for the various laboratory parameters was refrigerated overnight at 4°C and delivered to the laboratory for testing the morning following collection.

III. STATISTICAL ANALYSIS

The mean and standard deviation (mean ± SD) are reported for each parameter measured. A one way factorial analysis of variance (ANOVA) was used to compare groups. Both correlation coefficients and stepwise multiple regression analyses were used to assess relations between parameters. In all cases $p<.05$ was considered significant. The tables and figures indicate the exact number (N) of analyses in each statistical comparison.

RESULTS

Results of blood lipoproteins and blood counts in the total population of 982 and the population grouped by gender (43.5% males, 56.7% females) are shown in Table 1. There were no significant differences in age, total cholesterol, LDLc, or WBC between men and women enrolled in this study. HDLc was significantly higher in women (50.3 vs. 42.8 mg/dl, $p<0.001$). Triglycerides were significantly lower in women than in men (149.8 mg/dl vs. 207.1 mg/dl, $p<0.001$). Fibrinogen levels were higher and hematocrit and WBV lower in women than men (all $p<0.0001$).

There was a significant ($r = 0.78$, $p<0.001$) correlation between the packed cell volume (PCV) and the whole blood viscosity (Figure 1). There were significant, positive correlations of WBV with serum cholesterol ($r = 0.22$, $p<0.001$, Figure 2), serum triglycerides ($r = 0.14$, $p<0.001$, Table 2) and serum LDL cholesterol levels ($r = 0.21$, $p<0.001$, Figure 3). There was a significant, negative correlation between WBV and serum HDLc levels ($r = -0.20$, $p<0.001$, Figure 4).

When the studies were analyzed by gender (Table 2), the correlations were similar for all findings except for the negative overall correlation between HDLc and WBV was not observed in females, and the positive overall correlation between triglycerides and WBV was not observed in men.

DISCUSSION

We observed positive correlations between WBV and cholesterol, triglycerides and LDLc and a negative correlation between WBV and HDLc. When analyzed by gender, the correlations between WBV and LDLc and cholesterol were significant for both sexes, while the correlation between WBV and triglyceride was significant only for women. The explanation for the positive correlations observed between WBV and LDLc may lie in direct effects of the LDLc macromolecules on plasma viscosity as well as the enhancement of red blood cell interactions by large lipoprotein molecules. Previous studies have demonstrated that intrinsic red blood cell factors such as microcytosis or macrocytosis may enhance or diminish whole blood viscosity respectively (16,17), but these factors do not offer any explanation for the correlation between WBV and lipids observed in the present study. A previous study of patients on renal dialysis treated with erythropoietin (18) has shown that viscosity is decreased in proportion to the response to erythropoietin suggesting that younger red blood cells have intrinsically less viscosity than do older red blood cells. Moreover, Levy et al. (19) have demonstrated that platelet membrane stiffness is related to membrane lipoprotein concentration. LDLc is subject to oxidation (20) as are red blood cell membrane associated lipids; higher levels of cholesterol and LDLc may favour increased oxidation of the red blood cell membrane lipids. This process may lead to stiffening of the red blood cell membrane akin to the normal RBC aging process. Experimental studies of red blood cell membrane stiffness will be needed to verify whether this

explanation actually accounts for the correlation between increased WBV and increased LDLc.

Our results confirm previous studies that have shown a positive correlation between total cholesterol level and whole blood viscosity (4,5). Since apoprotein B concentration is highly correlated with total cholesterol, it is not surprising that LDLc correlated strongly with whole blood viscosity. Koenig et al. have recently reported that plasma viscosity is found to have a positive correlation with total cholesterol as well as apoprotein B (4). The r values for these variables for men and women were 0.23 and 0.24 respectively, similar to our values correlation coefficients of 0.24 for men and 0.25 for women between LDLc and WBV. Thus, our finding that LDLc and total cholesterol correlate significantly with whole blood viscosity may be at least explained partly by the observation that apolipoprotein B correlates with plasma viscosity.

As was previously demonstrated in a smaller group of normal individuals (15), there was a considerable variability in whole blood viscosity that was not predicted from hematocrit alone. The measured range of whole blood viscosity at a hematocrit of 45 vol%, for example, was more than twofold (6-14 mPa·sec., Figure 1). This was consistent with previous studies (5) in which approximately one-half of inter-individual variation of blood viscosity could be accounted for by the variation in the packed red blood cell volume. The residual variation in blood viscosity has not been explained. Plasma protein factors may act independently and in conjunction with red blood cell deformability to alter whole blood viscosity. The contribution of fibrinogen to whole blood viscosity has been reported (8). However, the conditions under

which fibrinogen affects WBV vary with shear rate and method used to measure whole blood viscosity (15). The present viscometer operates under disaggregating conditions at a shear rate of 19 inverse seconds which is relatively insensitive to detection of the effects of fibrinogen. Neither in this study nor in the previous study (15) was fibrinogen correlated with whole blood viscosity when measured in this manner. Variation in fibrinogen level did not account for the large inter-individual variation in whole blood viscosity not explained by the packed red blood cell volume. Fibrinogen levels did not explain the correlation between whole blood viscosity and cholesterol, LDLc and triglycerides.

High density lipoprotein cholesterol is associated with an improved outlook in coronary artery disease (21-23). Our finding of a negative correlation between HDLc and whole blood viscosity suggests the possibility that the protective effect of HDLc may be partially explained through its association with lower whole blood viscosity. The recent study of Koenig et al. also found a negative correlation between HDLc, total cholesterol and plasma viscosity (4). Moreover, a highly significant negative correlation between erythrocyte aggregation (EA) and high density lipoprotein cholesterol has been reported (24) for both normal individuals and patients with coronary artery disease. The method of measuring EA was based on a standardized test procedure using very low red blood cell concentrations achieved by dilution with autologous plasma. We found an overall negative correlation of HDLc and WBV, but when the data were analyzed by gender, this correlation, unlike the LDLc WBV correlation, was no longer significant. We think the most likely explanation to account for

the overall negative correlation between HDLc and WBV (Figure 4) is that HDLc levels are higher and WBV lower in women which causes a fortuitous negative association present only when all data are plotted but not found when the data were analyzed separately by sex (Table 1). In contrast, the positive correlation of LDLc and WBV was significant for the entire group (Figure 3) as well as for each gender separately (Table 1).

From an epidemiologic point of view, it seems reasonable to believe that individuals with high LDLc levels and high hematocrits are at greater risk for heart disease than individuals with only one of these risk factors (1,4). With respect to defining the contributory risk of a relatively increased WBV in such individuals, a prospective study of the effects of decreasing WBV (e.g. by phlebotomy) on the subsequent risk for cardiovascular disease in these individuals would need to be performed. Phlebotomy is a relatively simple, and inexpensive means to reduce WBV, and a pilot study of young patients with myocardial infarction and high LDLc levels is underway at this institution to determine the safety and efficacy of phlebotomy in this setting.

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Figure 1: The relation between whole blood viscosity and packed cell volume in a normal population.

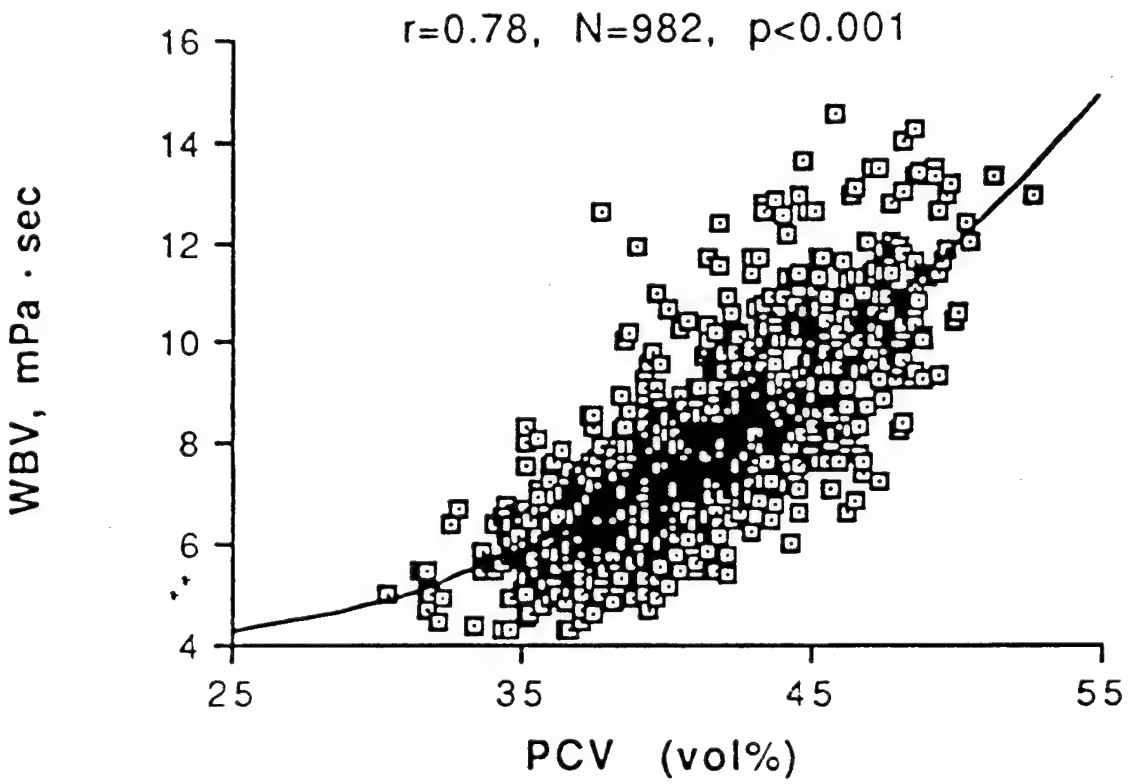

Figure 2: The relation between WBV and serum total cholesterol levels in a normal population.

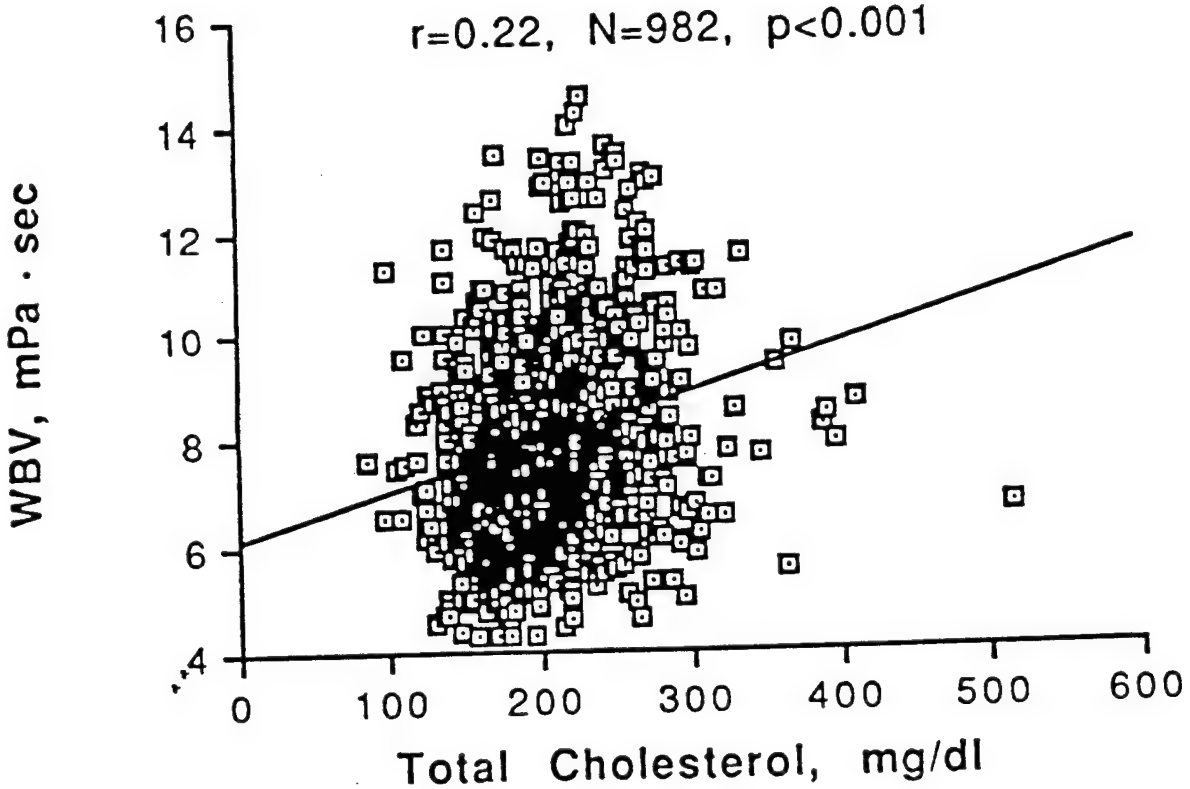

Figure 3: The relation between WBV and serum low density lipoprotein levels in a normal population.

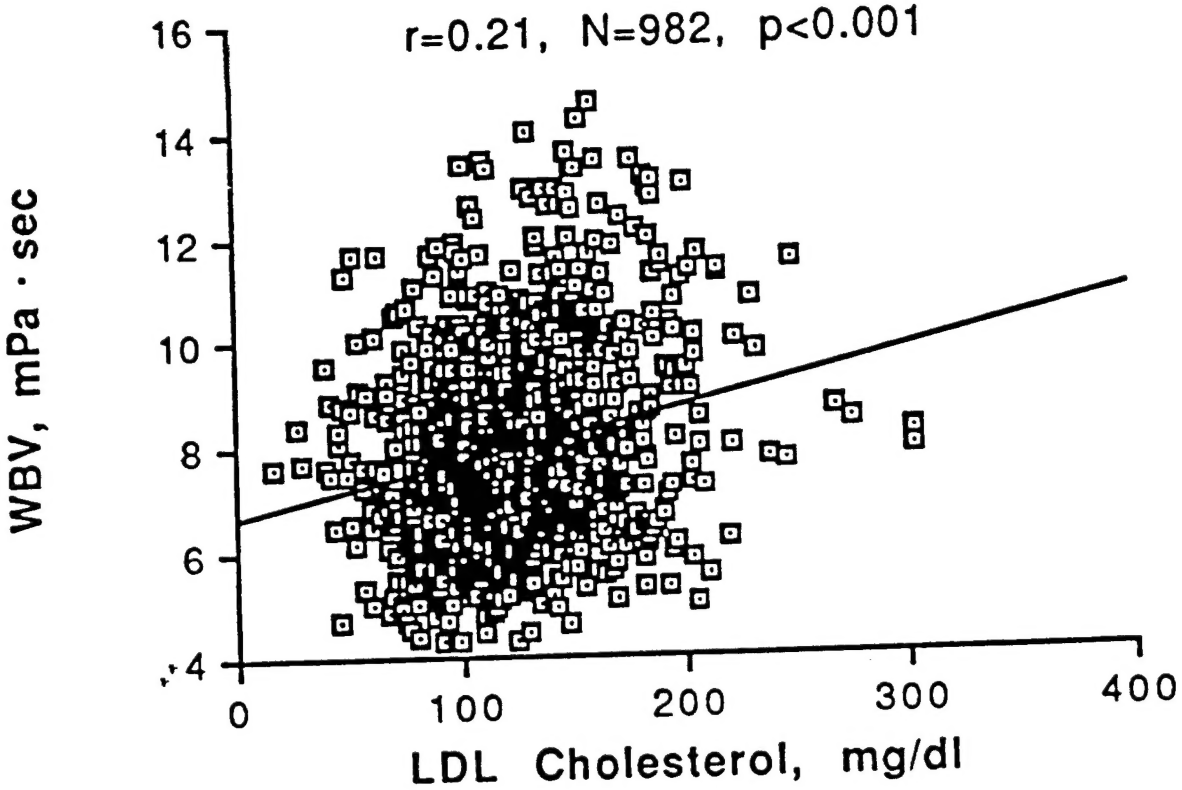

Figure 4: The relation between WBV and serum high density lipoprotein levels in a normal population.

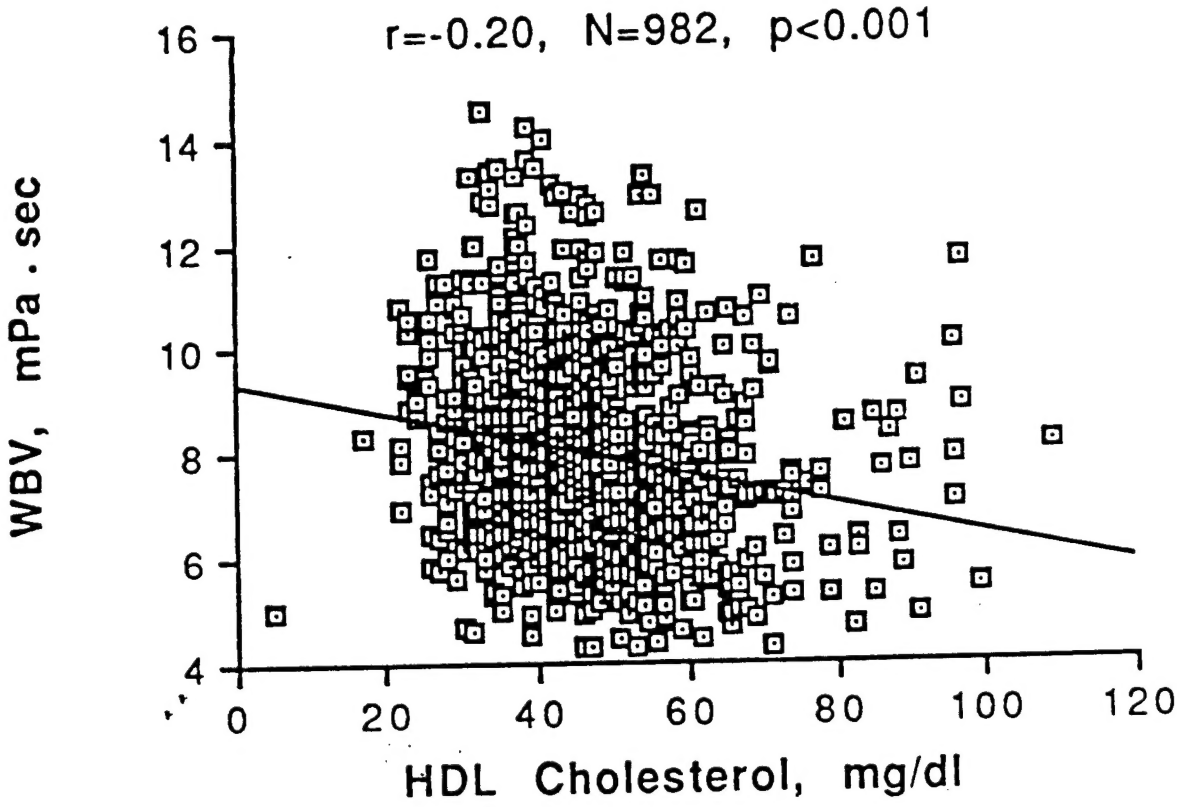

Table 1

PARAMETERS OF THE 982 NORMAL VOLUNTEERS BY TYPE AND GENDER

	1. ALL*	2. MALE	3. FEMALE	ANOVA (2 VS. 3)
NUMBER	982	425	557	---
AGE	41.3±14.5*	40.7±14.7	41.7±14.4	NS
CHOLESTEROL (mg/dl)	207.8±45.5	209.3±44.7	206.7±46.2	NS
HDL cholesterol (mg/dl)	47±13	42.8±11.8	50.3±13	0.001
LDL cholesterol (mg/dl)	125.9±37.9	124.7±39.5	126.8±36.7	NS
TRIGLYCERIDES (mg/dl)	174.6±156	207.1±135.3	149.8±166	0.001
WBC ($x10^6$/ml)	7.7±2.2	7.74±2.2	7.73±2.2	NS
HEMOGLOBIN (g/dl)	14±1.4	15±1.1	13.2±1.1	0.001
HEMATOCRIT (vol%)	41.3±3.9	44.1±3.1	39.1±2.9	0.001
PLATELET($x10^6$/ml)	277.8±68.2	269±64.5	284.6±70.2	0.001
FIBRINOGEN(mg/dl)	268.8±81.6	252.2±73.4	281.5±85.2	0.001
WBV mPa· sec	8.0±1.9	9.0±1.9	7.2±1.6	0.001

*Mean ± SD

Table 2

THE RELATION OF VARIOUS PARAMETERS TO WHOLE BLOOD VISCOSITY (WBV) BY LINEAR REGRESSION ANALYSIS

	All		**Males**		**Females**	
WBV vs	r	p	r	p	r	p
Cholesterol	0.22	.001	0.21	0.001	0.25	0.001
HDLc	-0.20	0.001	0.12	0.01	0.05	NS
LDLc	0.21	0.001	0.24	0.001	0.25	0.001
Triglycerides	0.14	.001	0.05	NS	0.08	0.05
WBC	0.20	.001	0.18	0.002	0.26	0.001
Hemoglobin	0.74	.001	0.66	0.001	0.65	0.001
Hematocrit	0.78	.001	0.72	0.001	0.70	0.001
Platelet	0.06	NS	0.11	0.02	0.13	0.002
Fibrinogen	0.01	NS	0.12	0.01	0.08	NS